Journal of Cardiology (2012) 59, 182—189

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Original article

Association of plasma cystatin C levels with angiographically documented coronary artery disease in patients of Indian origin

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Received 1 September 2011; received in revised form 27 October 2011; accepted 30 November 2011
Available online 21 January 2012

KEYWORDS
Coronary artery disease;
Kidney;
Angiography;
Cystatin C

Summary
Background: Renal impairment in patients with coronary artery disease (CAD) is common and increases morbidity and mortality. Estimation of glomerular filtration rate (GFR) by measuring serum creatinine (Cr) or Cr clearance has limitations. Cystatin C is a novel marker for renal function that is very sensitive and specific for GFR estimation. The utility of plasma cystatin C (PCyC) in patients with CAD needs further study, especially in the developing world, where CAD is rising exponentially.
Methods and results: In a prospective study of 150 patients undergoing coronary angiography, median PCyC was 1.45 mg/L; patients with levels ≥1.45 mg/L were older, had higher mean number of diseased coronary vessels, more frequently had triple vessel disease (TVD), and diffuse CAD on angiography. This association of higher PCyC levels with CAD remained robust even after excluding patients with eGFR < 60 ml/min/1.73 m^2. The relative risk (RR) of having TVD or diffuse CAD in the overall cohort was 1.7 and 1.9, while it was 1.91 and 2.3 respectively in those with eGFR ≥ 60 ml/min/1.73 m^2, with PCyC levels more than median. Categorization of the entire cohort and those with eGFR ≥ 60, into tertiles based on 33rd and 66th percentiles of PCyC maintained the association of cystatin C with more severe CAD.
Conclusion: In Indian patients with CAD, higher PCyC levels are associated with more severe CAD. The association of PCyC with severe CAD remains robust even in patients with normal or mildly impaired renal function. Cystatin C may have potential clinical usefulness as a marker for identification of high risk CAD patients.

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0914-5087/$ — see front matter
doi:10.1016/j.jjcc.2011.11.013

Introduction

Concomitant chronic renal impairment (RI) is frequent in patients with cardiovascular disease and substantially increases morbidity and mortality [1—5]. Early identification of patients with RI is important to reliably detect high-risk patients and institute reno-protective measures whenever required. Traditionally, RI has been diagnosed by indirectly calculating glomerular filtration rate (GFR) either by serum creatinine (Cr) levels or estimation of creatinine clearance (CrCl) by Cockcroft-Gault and Modification of Diet in Renal Disease (MDRD) equations [6,7]. However assessment of GFR using these has several limitations, since Cr may not be elevated in mild RI, while CrCl often overestimates true GFR [8]. Hence a more sensitive and robust marker of RI is required in clinical practice.

Cystatin C, a cysteine protease inhibitor, has been proposed as a more sensitive and specific marker for detection of RI, and fulfills many criteria of an ideal marker of GFR [9]. It is less influenced by age, gender, race, muscle mass, and medication, as compared to serum Cr and is sensitive to even small changes in GFR, making it a superior marker to detect mild RI which may not be detected by routine Cr measurements [10,11].

Earlier studies have shown a close relationship between plasma cystatin C (PCyC) and various subsets of atherosclerotic disease including peripheral as well as coronary artery disease (CAD), both with stable CAD as well as those with acute coronary syndromes (ACS) [12—16]. However, a few studies in patients with CAD have failed to show correlation between PCyC with CAD and atherosclerosis [17—19]. Hence the clinical utility of cystatin C in patients with manifest CAD, especially with normal or only mild RI merits further investigation.

Moreover despite the increasing trends of CAD and RI in the developing world, scant data are available on PCyC levels and its predictive value in patients with angiographically proven CAD in patients of Indian origin.

Methods

Patients with suspected or proven CAD undergoing coronary angiography at our institute, were included in this cross-sectional observational study, which conformed to the institutional ethical guidelines. Patients were enrolled in the study with prior written informed consent; all patients underwent routine biochemical tests prior to coronary angiography, while PCyC levels were measured using particle-enhanced nephelometric immunoassay (PENIA) method (*N* Latex Cys-C, Dade Behring, Deerfield, IL, USA). The estimated (e) GFR was calculated from MDRD study equation: $(ml/min/1.73\,m^2) = 175 \times (Scr)^{-1.154} \times (Age)^{-0.203} \times (0.742$ if female$) \times (1.210$ if African American). Hypertension (HT) was defined as blood pressure $\geq$140/90 mmHg or having a history of antihypertensive drug use. The subjects actively inhaling tobacco smoke in any form were considered smokers. Diabetes mellitus (DM) was defined as fasting blood glucose level $\geq$100 mg/dl or having a history of oral hypoglycemic drug or insulin use.

Patients with significant valvular or other structural heart disease, severe symptomatic heart failure, life-threatening arrhythmias, acute and chronic liver disease, infectious, auto-immune and chronic inflammatory disease, cancers, and on any form of renal replacement therapy, were excluded.

All patients also underwent assessment of carotid intimal medial thickness (CIMT) which was measured as per standard protocol with 10 MHz linear vascular probe on GE Vivid 7 dimension machine (GE Healthcare, Chalfont St Giles, UK). The carotid segments used for measurement of CIMT were the distal straight 1 cm of the common carotid arteries, the carotid bifurcations, and the proximal 1 cm of the internal carotid arteries. The average of measurements taken during 3 cardiac cycles at end-diastole and the average of right and left CIMT were taken as the mean CIMT.

Coronary angiography was performed in the standard manner in all the patients through either radial or femoral artery access. Angiographic analysis was done by standard quantitative angiographic techniques and lesion length quantified by measuring precisely from normal to normal segment. Angiographic CAD was defined as >50% of diameter stenosis in any of the major epicardial coronary arteries, while diffuse CAD was defined as involvement of >20 mm segment in a particular epicardial vessel. For exact evaluation of a tandem lesion, we followed the principle that if multiple lesions are less than 3 vessel reference diameters apart (true tandem lesion), these lesions would be scored as one lesion.

Statistical analysis

All the data were analyzed using SPSS .16 statistical software (SPSS Inc., Chicago, IL, USA). All data are expressed as mean $\pm$ standard deviation. Student *t*-test was used to compare means between groups, and the chi-square test was used to compare proportions between groups. Demographic and clinical characteristics were compared across 3 categories of PCyC level with analysis of variance (ANOVA). The Pearson correlation was used to analyze the correlations between diffuse CAD with cystatin C and other risk factors; *p*-value less than 0.05 was considered statistically significant.

Results

The demographics and clinical features of the patient population are summarized in Table 1. The enrolled 150 patients are from among patients undergoing coronary angiography at our institute from the period of October—December 2011. Of 166 patients screened, 16 were excluded (11 due to being on renal replacement therapy, and 5 having significant valvular heart disease). The mean age was 57.89 $\pm$ 9.43 years and 86% were males. HT was present in 53%, DM in 36%, and history of smoking in 33% of cases. Chronic stable angina was noted in 33% while a history of ACS (within the last 4 weeks) was present in 60%. The mean serum Cr of the cohort was 1.14 $\pm$ 0.56 (0.64—5.59) mg/dl while mean eGFR was 70.97 $\pm$ 18.86 (11.25—114.38) $ml/min/1.73\,m^2$. Forty of one hundred and fifty (26%) patients had significant renal impairment (eGFR < 60 $ml/min/1.73\,m^2$);

Table 1 Baseline characteristics of the entire cohort (150 patients).

Variables	Mean ± SD (*N* = 150)
Age (years)	57.89 ± 9.43
Sex (M:F)	128:22
STEMI [*N*(%)]	41 (27%)
USA/NSTEMI [*N*(%)]	49 (33%)
CSA [*N*(%)]	49 (33%)
LVEF (%)	51.02 ± 10.89
TG [mg%]	135.41 ± 58.04
TC [mg%]	135.40 ± 40.15
HDL [mg%]	32.78 ± 8.73
LDL [mg%]	75.44 ± 32.96
eGFR (MDRD) (ml/min/1.73 m^2)	70.97 ± 18.86
Serum creatinine (mg/dl)	1.14 ± 0.56
CIMT (mean) (mm)	0.63 ± 0.11
Cystatin C (mg/L)	1.8 ± 0.72
SVD [*N*(%)]	37 (24.5%)
DVD [*N*(%)]	35 (23.2%)
TVD [*N*(%)]	59 (39.1%)
No/Insignificant disease [*N*(%)]	20 (13.2%)
HT [*N*(%)]	80 (53%)
DM [*N*(%)]	54 (35.8%)
Smoker [*N*(%)]	49 (32.7%)
Metabolic syndrome [*N*(%)]	90 (60%)

All values are expressed as mean ± SD.
STEMI, ST elevation myocardial infarction; USA, unstable angina; NSTEMI, non ST-elevation myocardial infarction; CSA, chronic stable angina; LVEF, left ventricle ejection fraction, as measured by echocardiography; TG, serum triglycerides; TC, serum total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; eGFR (MDRD), estimated glomerular filtration rate by 4 component Modification of Diet in Renal Disease formula; CIMT, carotid intima media thickness; SVD, single vessel disease; DVD, double vessel disease; TVD, triple vessel disease; HT, hypertension; DM, diabetes mellitus.

of these 37 had eGFR 30—60 ml/min/1.73 m^2 while 3 had eGFR < 30 ml/min/1.73 m^2. Mean PCyC levels were 1.8 ± 0.72 (0.51—6.87) mg/L; patients with eGFR < 60 ml/min/1.73 m^2 had significantly higher mean PCyC levels (2.11 ± 1.11 mg/L) as compared to those with eGFR ≥ 60 ml/min/1.73 m^2 (1.36 ± 0.35 mg/dl, $p < 0.001$). Coronary angiography revealed normal coronary anatomy or insignificant CAD in 13%, while single, double, and triple vessel (TVD) CAD were present in 25%, 23%, and 39% respectively. In the final analysis, the 20 patients with no significant CAD were excluded.

Analysis of the entire patient cohort according to cystatin C level

The median PCyC levels of the entire cohort was 1.45 mg/L. Categorizing the patients into two groups according to PCyC levels ≥/<1.45 mg/L revealed that patients with higher PCyC levels were older (60.3 ± 8.9 years vs. 55.4 ± 9.4 years, $p < 0.001$), had higher serum Cr (1.32 ± 0.73 mg/dl vs. 0.95 ± 0.16 mg/dl, $p < 0.001$), lower eGFR (61.59 + 18.97 ml/min/1.73 m^2 vs. 80.74 ± 12.91 ml/min/1.73 m^2, $p < 0.001$), and higher mean CIMT (0.64 + 0.11 mm vs. 0.61 + 0.10 mm, $p < 0.05$) (Table 2). Analysis of angiographic severity of CAD in these two subgroups revealed that patients with higher PCyC levels more frequently had TVD on coronary angiography (46% vs. 32%, $p = 0.07$) and diffuse angiographic CAD (69% vs. 54%, $p = 0.04$). The mean number of diseased coronary vessels demonstrated a trend toward being higher in those with PCyC levels ≥1.45 mg/dl (2.03 ± 1.05 mg/dl vs. 1.73 ± 1.09 mg/dl), though this was of only borderline statistical significance ($p = 0.06$, Fig. 1). The prevalence of HT, DM, smoking and clinical pattern of presentation of CAD were similar among the two groups.

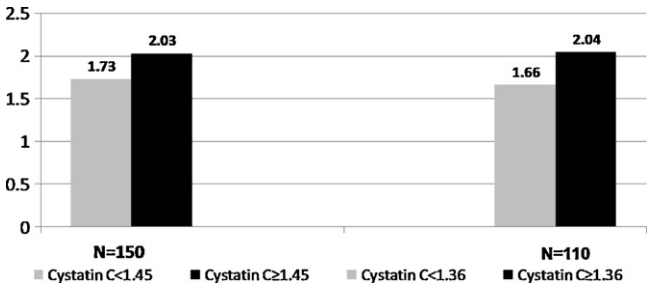

Figure 1 Mean number of diseased coronary vessels involved in different groups.

Relative risk of developing TVD or diffuse CAD according to PCyC levels

Among the entire patient cohort, patients with PCyC ≥ 1.45 mg/dl had a 1.7 times higher relative risk (RR) of having TVD (95% CI 0.89—3.36) and a 1.9 times higher RR of having diffuse CAD on coronary angiography (95% CI 0.96—3.65, $p = 0.04$).

Division of the entire patient cohort into tertiles based on 33rd and 66th percentiles of PCyC levels

We observed that compared to patients in the lower two tertiles (PCyC < 1.32 mg/L and 1.32—1.61 mg/L), those in the highest tertile (PCyC > 1.61 mg/L) were significantly older (60.5 ± 9.1 years, 58.5 ± 9.2 years, and 54.8 ± 9.2 years, respectively, $p = 0.007$) (Table 3); had higher mean serum Cr levels (1.42 ± 0.88 mg/dl, 1.06 ± 0.21 mg/dl, and 0.94 ± 1.67 mg/dl, respectively, $p = 0.001$), lower mean eGFR (58.3 ± 19.5 ml/min/1.73 m^2, 72.3 ± 16.3 ml/min/1.73 m^2, and 81.74 ± 12.3 ml/min/1.73 m^2, respectively, $p = 0.001$), and higher mean CIMT (0.65 ± 0.12 mm, 0.62 ± 0.11 mm, and 0.60 ± 0.09 mm, respectively, $p < 0.05$). The incidence of HT, DM, smoking and mode of presentation of CAD were similar among the three groups. Coronary angiography revealed that those in the highest PCyC tertile had significantly more frequent occurrence of diffuse CAD (72%, 64%, and 49%, $p = 0.05$), and a trend toward having more frequent TVD (48%, 37%, and 32%, $p = 0.07$) (Fig. 2). A rising trend of having higher mean number of diseased coronary arteries was also observed in those in the highest PCyC tertile (2.14 + 1.06, 1.88 + 1.06 and

Table 2 Analysis of the entire patient cohort according to median cystatin C levels.

Variables	Cystatin C <1.45 (mg/L)	Cystatin C ≥1.45 (mg/L)	*p*-Value
Age (years)	55.4 ± 9.4	60.3 ± 8.9	0.001
Sex (M:F)	62:12	67:10	NS
STEMI [*N*(%)]	20 (27%)	21 (27.6%)	NS
USA/NSTEMI [*N*(%)]	23 (31%)	26 (34.2%)	NS
CSA [*N*(%)]	24 (32.4%)	25 (32.9%)	NS
LVEF (%)	50.79 ± 11.5	51.24 ± 10.35	NS
Serum creatinine (mg/dl)	0.95 ± 0.16	1.32 ± 0.73	<0.001
eGFR (MDRD) (ml/min/1.73 $m^{2)}$	80.74 ± 12.91	61.59 ± 18.97	<0.001
CIMT (mean) (mm)	0.61 ± 0.10	0.64 ± 0.110	0.05
TVD [*N*(%)]	24 (32.4%)	35 (45.5%)	0.07
Diffuse CAD [*N*(%)]	40 (54.1%)	53 (68.8%)	0.04
HT [*N*(%)]	35 (47.3%)	45 (58.4%)	NS
DM [*N*(%)]	26 (35.1%)	28 (36.4%)	NS
Smoker [*N*(%)]	26 (35.1%)	23 (30.3%)	NS
Metabolic syndrome [*N*(%)]	46 (62.2%):28 (37.8%)	44 (57.1%):33 (42.9%)	NS
Mean no. of diseased vessels	1.73 ± 1.09	2.03 ± 1.05	0.06

Values are expressed as mean ± SD.
STEMI, ST elevation myocardial infarction; USA, unstable angina; NSTEMI, non ST-elevation myocardial infarction; CSA, chronic stable angina; LVEF, left ventricle ejection fraction, as measured by echocardiography; eGFR (MDRD), estimated glomerular filtration rate by 4 component Modification of Diet in Renal Disease formula; CIMT, carotid intima media thickness; TVD, triple vessel disease; CAD, coronary artery disease; HT, hypertension; DM, diabetes mellitus.

1.74 + 1.07, respectively), though this did not achieve statistical significance. The RR of developing TVD was 1.96 (95% CI 0.88—4.35) and RR of having diffuse CAD on coronary angiography was 2.67 (95% CI 1.18—6.06, $p = 0.01$) in those in the highest vs. the lowest PCyC tertile.

In the overall cohort, factors predicting diffuse CAD on univariate regression analysis included diabetes [odds ratio (OR) 3.68, 95% CI 1.69—7.96, $p < 0.001$], high-density lipoprotein cholesterol (HDL-C) levels (OR 0.95, 95% CI 0.91—0.98, $p = 0.013$) and PCyC levels (OR 1.65 95% CI 1.09—2.48, $p < 0.01$). Factors predicting diffuse CAD on multivariate regression analysis included diabetes and PCyC levels. Importantly, even after adjusting for diabetes, PCyC levels remained a significant predictor of diffuse CAD on multivariate regression analysis (OR 1.63, 95% CI 1.06—2.51, $p = 0.027$).

In the overall patient population, a significant positive correlation was observed between diffuse CAD and PCyC levels ($r = 0.195$, $p = 0.016$) and presence of DM ($r = 0.277$; $p = 0.001$) while a negative correlation was observed with HDL-C levels ($r = -0.21$; $p = 0.01$).

Table 3 Comparison of three tertiles based on 33rd and 66th percentiles of plasma cystatin C levels in entire cohort.

Variables	Cystatin C <1.32 mg/L	Cystatin C 1.32—1.61 mg/L	Cystatin C >1.61 mg/L	*p*-Value
Age (years)	54.8 ± 9.2	58.5 ± 9.2	60.5 ± 9.1	0.007
Sex (M:F)	45:8	41:7	43:7	NS
LVEF (%)	49.9 ± 11.5	51.8 ± 10.3	51.4 ± 10.7	NS
Serum creatinine (mg/dl)	0.94 ± 1.67	1.06 ± 0.21	1.42 ± 0.88	<0.001
eGFR (MDRD) (ml/min/1.73 m^2)	81.74 ± 12.3	72.3 ± 16.3	58.3 ± 19.5	<0.001
CIMT (mean) (mm)	0.60 ± 0.09	0.62 ± 0.11	0.65 ± 0.12	0.05
TVD [*N*(%)]	17 (32.1%)	18 (37.5%)	24 (48%)	0.07
Diffuse CAD [*N*(%)]	26 (49.1%)	31 (64.6%)	36 (72%)	0.05
HT [*N*(%)]	22 (41.5%)	27 (56.3%)	31 (62%)	0.09
DM [*N*(%)]	17 (32.1%)	21 (43.8)	16 (32%)	NS
Smoker [*N*(%)]	19 (35.8%)	16 (33.3%)	14 (28.6%)	NS
Metabolic syndrome [*N*(%)]	29 (54.7%)	30 (62.5%)	31 (62%)	NS
Mean no. of diseased vessels	1.74 + 1.07	1.88 + 1.06	2.14 + 1.06	0.07

Values are expressed as mean ± SD.
LVEF, left ventricle ejection fraction, as measured by echocardiography; eGFR (MDRD), estimated glomerular filtration rate by 4 component Modification of Diet in Renal Disease formula; CIMT, carotid intima media thickness; TVD, triple vessel disease; CAD, coronary artery disease; HT, hypertension; DM, diabetes mellitus.

Table 4 Analysis of patients with eGFR $\geq$ 60 ml/min/1.73 m^2 according to median cystatin C levels (n = 110).

Variables	Cystatin C <1.36 mg/L	Cystatin C $\geq$1.36 mg/L	p-Value
Age (years)	54.7 $\pm$ 9.3	57.89 $\pm$ 8.83	0.06
Sex (M:F)	46:10	50:5	NS
STEMI [N(%)]	17 (30.4%)	13 (23.6%)	NS
USA/NSTEMI [N(%)]	19 (30.3%)	17 (30.9%)	NS
CSA [N(%)]	17 (30.4%)	22 (40.0%)	NS
LVEF (%)	49.62 $\pm$ 11.36	53.41 $\pm$ 9.7	NS
Serum creatinine (mg/dl)	0.92 $\pm$ 0.13	0.99 $\pm$ 0.12	0.004
eGFR (MDRD) (ml/min/1.73 m^2)	82.65 $\pm$ 10.87	77.03 $\pm$ 11.78	0.01
CIMT (mean) (mm)	0.59 $\pm$ 0.10	0.62 $\pm$ .10	NS
TVD [N(%)]	17 (30.4%)	25 (45.5%)	0.07
Diffuse CAD [N(%)]	29 (51.8%)	39 (70.9%)	0.03
HT [N(%)]	23 (41.1%)	36 (65.5)	0.008
DM [N(%)]	19 (33.9%)	16 (29.1%)	NS
Smoker [N(%)]	18 (32.1%)	18 (32.7%)	NS
Metabolic syndrome [N(%)]	31 (55.4%)	32 (58.2%)	NS
Mean no. of diseased vessels	1.66 $\pm$ 1.1	2.04 $\pm$ 1.04	0.05

Values are expressed as mean $\pm$ SD.
STEMI, ST elevation myocardial infarction; USA, unstable angina; NSTEMI, non ST-elevation myocardial infarction; CSA, chronic stable angina; LVEF, left ventricle ejection fraction, as measured by echocardiography; eGFR (MDRD), estimated glomerular filtration rate by 4 component Modification of Diet in Renal Disease formula; CIMT, carotid intima media thickness; TVD, triple vessel disease; CAD, coronary artery disease; HT, hypertension; DM, diabetes mellitus.

Analysis in patients with eGFR $\geq$ 60 ml/min/1.73 m^2

Normal or near normal renal function (eGFR $\geq$ 60 ml/min) was present in 110/150 (74%) patients; the median PCyC levels in these patients were 1.36 mg/L. Categorizing these patients into two groups based on the median PCyC levels ($\geq$/$\leq$1.36 mg/L, Table 4) revealed that those with higher PCyC levels had significantly higher serum Cr levels (0.99 $\pm$ 0.12 mg/dl vs. 0.92 $\pm$ 0.13 mg/dl, p < 0.004), lower eGFR (77.03 $\pm$ 11.78 ml/min/1.73 m^2 vs. 82.65 $\pm$ 10.87 ml/min/1.73 m^2, p = 0.01) and more frequently had HT (66% vs. 41%, p = 0.008). Similar to what was observed in the overall cohort, even among these patients with normal/near normal eGFR, those with higher PCyC levels had significantly more frequent occurrence of diffuse angiographic CAD (71% vs. 51%, p < 0.03) and a trend toward having more frequent occurrence of TVD on coronary angiography (46% vs. 30%, p = 0.07). The mean number of diseased coronary vessels was also significantly higher in those with PCyC levels $\geq$1.36 mg/L as compared to those with lower levels (2.04 $\pm$ 1.04 vs. 1.66 $\pm$ 1.1, p = 0.05) (Fig. 1). The incidence of DM, smoking, and mode of clinical presentation of CAD were similar in the two groups.

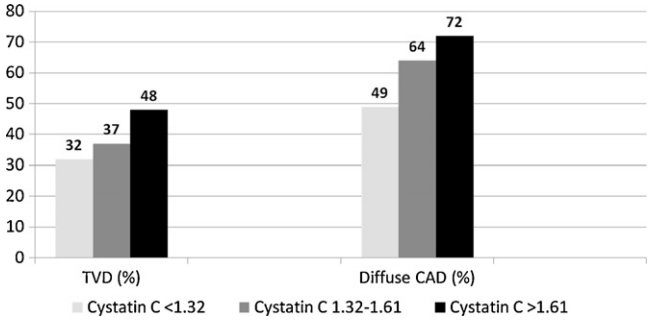

Figure 2 Triple vessel disease (TVD, %) and diffuse coronary artery disease (CAD, %) in plasma cystatin C (PCyC) tertiles (N = 150).

RR of developing TVD or diffuse CAD according to PCyC levels in those with normal/near normal eGFR

Excluding patients with eGFR < 60 ml/min/1.73 m^2 not only preserved but enhanced the association of the increased RR of angiographically documented TVD (RR 1.91, 95% CI 0.88—4.16) with PCyC $\geq$ 1.36 mg/L. The RR of having diffuse angiographically documented CAD was also higher as compared to that observed in the overall patient cohort (RR of 2.3, 95% CI 1.04—4.96, p = 0.03 with PCyC $\geq$ 1.36 mg/L).

Division of the patients with normal/near normal eGFR into tertiles based on 33rd and 66th percentiles of PCyC levels

Compared to patients in the lower two tertiles (i.e. PCyC levels <1.22 mg/L and 1.22—1.5 mg/L), those in the highest tertile (i.e. PCyC > 1.5 mg/L) had higher serum Cr levels (1.01 $\pm$ 0.12 mg/dl, 0.96 $\pm$ 0.11 mg/dl, and 0.91 $\pm$ 0.14 mg/dl, p = 0.007) and lower mean eGFR (75.8 $\pm$ 11.4 ml/min/1.73 m^2, 79.7 $\pm$ 11.2 ml/min/1.73 m^2, and 84.2 $\pm$ 11.1 ml/min/1.73 m^2, p = 0.009) (Table 5). However mean age and CIMT were comparable among the three tertiles, in contrast to what was observed during analysis of

Table 5 Comparison of three tertiles based on 33rd and 66th percentiles of plasma cystatin C levels in patients with eGFR (MDRD) $\geq$60 ml/min/1.73 m^2 (n = 110).

Variables	Cystatin C <1.22 (mg/L)	Cystatin C 1.22—1.5 (mg/L)	Cystatin C >1.5 (mg/L)	p-Value
Age (years)	53.9 ± 8.7	57.1 ± 9.5	57.7 ± 8.9	0.172
Sex (M:F)	29:6	35:6	32:3	0.557
LVEF (%) [mean ± SD]	49.2 ± 12.3	51.4 ± 10.5	53.9 ± 8.8	0.183
Serum creatinine (mg/dl)	0.91 ± 0.14	0.96 ± 0.11	1.01 ± 0.12	0.007
eGFR (MDRD) (ml/min/1.73 m^2)	84.2 ± 11.1	79.7 ± 11.2	75.8 ± 11.4	0.009
CIMT (mean) (mm)	0.61 ± 0.11	0.60 ± 0.10	0.63 ± 0.10	NS
TVD [*N*(%)]	13 (31.7%)	13 (37.1%)	16 (45.7%)	0.06
Diffuse CAD [*N*(%)]	19 (54.3%)	25 (61%)	24 (68.6%)	0.06
HT [*N*(%)]	14 (40%)	22 (53.7%)	23 (65.7%)	0.09
DM [*N*(%)]	11 (31.4%)	17 (41.5%)	7 (20%)	NS
Smoker [*N*(%)]	12 (34.3%)	13 (31.7%)	11 (31.4%)	NS
Metabolic syndrome [*N*(%)]	19 (54.3%)	25 (61%)	19 (54.3%)	NS
Mean no. of diseased vessels	1.76 + 1.04	1.80 + 1.15	2.01 + 1.05	NS

Values are expressed as mean ± SD.
LVEF, left ventricle ejection fraction, as measured by echocardiography; eGFR (MDRD), estimated glomerular filtration rate by 4 component Modification of Diet in Renal Disease formula; CIMT, carotid intima media thickness; TVD, triple vessel disease; CAD, coronary artery disease; HT, hypertension; DM, diabetes mellitus.

the entire patient cohort. The prevalence of HT, DM, smoking, and clinical pattern of presentation of CAD were similar among the three groups. On coronary angiography, those in the highest PCyC tertile had a trend toward more frequent occurrence of diffuse CAD (69%, 61%, and 54% respectively) as well as TVD (46%, 37%, and 31%) although this did not achieve statistical significance (Fig. 3). There was also a trend toward having higher mean number of diseased coronary arteries among those in the highest PCyC tertile (2.01 + 1.05, 1.80 + 1.15, and 1.76 + 1.04 respectively). Even in patients with normal/near normal renal function (eGFR $\geq$ 60 ml/min/1.73 m^2), there was an increased RR of developing TVD as well as diffuse CAD with rising PCyC levels (RR of developing TVD: 1.4, 95% CI 0.55—3.7 and RR of having diffuse CAD: 1.88, 95% CI 0.69—4.87) in the highest vs. the lowest PCyC tertiles respectively.

In these patients with near normal eGFR, factors predicting diffuse CAD on univariate regression analysis included diabetes (OR 4.59, 95% CI 1.71—12.31, p = 0.003), and PCyC levels (OR 2.27, 95% CI 1.04—4.97, p = 0.04). Factors predicting diffuse CAD on multivariate regression analysis included DM and PCyC levels. Importantly, even after adjusting for DM, PCyC levels remained a significant predictor of diffuse CAD on multivariate regression analysis [OR = 2.69 (95% CI 1.16—6.21, p = 0.021)].

Similar to the overall patient cohort, a significant positive correlation was observed between diffuse CAD and PCyC levels (r = 0.196, p = 0.039) and DM (r = 0.301, p = 0.01).

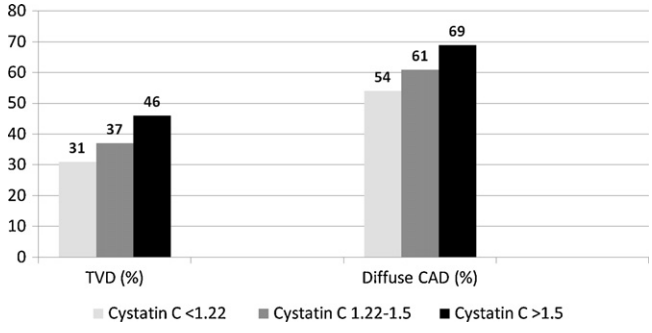

Figure 3 Triple vessel disease (TVD, %) and diffuse coronary artery disease (CAD, %) in plasma cystatin C (PCyC) tertiles (N = 110).

Discussion

Concomitant chronic kidney disease in patients with CAD is common and substantially increases the morbidity and mortality. Although even mild RI is associated with an increased cardiovascular risk, due to the nonlinear relationship between Cr levels and GFR, Cr is unreliable for detecting small reductions in GFR and mild RI [1,10,20,21]. Cystatin C is novel marker for renal function that has been shown to be more sensitive and specific for GFR estimation than Cr-based formulas. It is less influenced by age, gender, race, drugs, and muscle mass as compared to Cr and estimation of PCyC levels is known to be a better indicator of mild RI which may not be detectable by Cr measurement [11,22].

In our prospective study of 150 patients undergoing coronary angiography, we observed that patients with higher absolute PCyC levels were slightly older, had higher CIMT, more frequently had angiographically documented TVD and diffuse CAD and also had a trend toward having higher mean number of diseased coronary vessels as compared to those with lower PCyC levels. This association of higher PCyC levels with CAD remained robust even in those patients with normal/near normal eGFR (eGFR > 60 ml/min/1.73 m^2). Interestingly, in these patients with supposedly normal eGFR, those with PCyC levels higher than the median value had significantly lower eGFR as compared to those with lower PCyC levels (Figs. 4 and 5). Among patients with normal/near normal renal function we again observed that those with higher absolute PCyC levels had more frequent occurrence of TVD, higher prevalence of diffuse

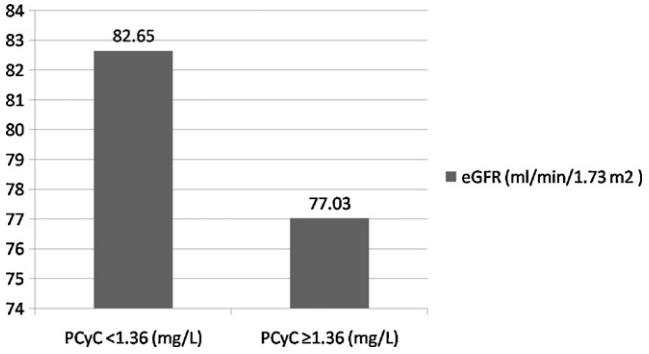

Figure 4 Mean estimated glomerular filtration rate (eGFR) in 110 patients with eGFR (Modification of Diet in Renal Disease) $\geq$60 ml/min/1.73 m^2 according to median plasma cystatin C (PCyC) levels.

angiographic CAD, and significantly higher mean number of diseased coronary vessels as compared to those with lower PCyC levels.

Enhanced RR of angiographic CAD with higher absolute PCyC levels

In the overall patient cohort, the RR of having TVD and diffuse CAD was 1.7 and 1.9 respectively, in those with high absolute PCyC levels. Among patients with normal/near normal renal function, this association of severe CAD with higher PCyC levels was not only preserved, but was further enhanced (RR of TVD 1.91, RR of diffuse CAD 2.3).

Previous studies have also shown a close relationship between cystatin C and CAD both in patients with stable CAD as well as those with ACS and ST elevation myocardial infarction [14,16,23,24]. The European Society of Cardiology has recommended the use of cystatin C for predicting myocardial infarction and long-term mortality in patients with non-ST elevation ACS [25]. Elevated levels of PCyC have been shown to be superior to serum Cr, eGFR, or CrCl in predicting the occurrence and severity of CAD as well as cardiovascular events and outcome during follow up [13,23,24,26—28]. However some other studies have failed to demonstrate an association between PCyC levels and CAD and atherosclerosis [17—19].

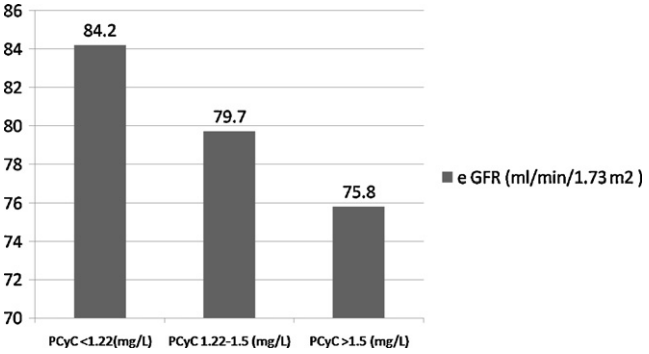

Figure 5 Mean estimated glomerular filtration rate (eGFR) in three plasma cystatin C (PCyC) tertiles based on 33rd and 66th percentiles of PCyC levels in patients with eGFR (Modification of Diet in Renal Disease) $\geq$60 ml/min/1.73 m^2 (N = 110).

Categorization of the entire patient cohort as well as those with eGFR > 60 ml/min/1.73 m^2 into tertiles based on 33rd and 66th percentiles of PCyC revealed that expectedly, patients in the highest cystatin tertiles had significantly higher mean serum Cr and lower mean eGFR values. The coronary angiography profile was also worse in those in the highest PCyC tertile; these patients had higher mean number of diseased coronary arteries, more frequent occurrence of TVD, and more frequent presence of diffuse CAD as compared to those in the lower two tertiles.

Enhanced RR of angiographic CAD within PCyC tertiles

Similar to the trends seen with higher absolute levels of PCyC, there was a higher RR of TVD (1.96) and diffuse CAD (2.67) in the highest vs. the lowest PCyC tertiles; the association of severe CAD with higher PCyC tertiles was preserved in those with normal/near normal renal function (RR of TVD 1.4, RR of diffuse CAD 1.88).

A rising trend of more severe CAD and worse clinical outcomes with higher PCyC levels has been reported previously [14,23,24,29]. In patients with non-ST-elevation ACS, the RR of death during follow up was 1.8, 3.2, and 11.7 in the 2nd, 3rd, and 4th quartiles of cystatin C compared with the lowest quartile, and the highest risk was observed in those with levels >1.25 mg/L [14]. Koc et al. [23] in a study of 94 stable patients with CAD reported that CAD severity score increased as the PCyC quartile increased (quartiles < 0.65 mg/L, 0.65—0.82 mg/L, 0.83—99 mg/L, and >1 mg/L). Keller et al. [29] reported that across the spectrum of CAD, patients in the highest cystatin C quartile had a 3.87 fold higher risk of cardiovascular death as compared to the pooled lower quartiles. Sekizuka et al. [30] also reported that serum cystatin C was significantly greater in multivessel CAD and suggested that PCyC levels may be considered a novel risk factor for coronary arteriosclerosis. The association of cystatin C with cardiovascular risk is postulated to be due to either direct pathologic effects, or due to heightened inflammatory state and clustering with other risk factors, all leading to enhanced vulnerability of the atherosclerotic plaque [13,31]. Whatever be the mechanism, our study also supports the observation that higher cystatin C levels are associated with more severe CAD, even in the patient subset with normal/near normal renal function.

Conclusion

Higher levels of cystatin C in patients with more severe CAD suggest its clinical usefulness as a potential biomarker for identification of high risk CAD patients. Whether this would assume further importance with an increasing burden of coexisting subclinical chronic kidney disease and cardiovascular disease as the patient population becomes older, needs to be substantiated in larger studies.

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